BULETIN VETERINER UDAYANA

pISSN 2085-2495

eISSN 2477-2712

Received: 24 January 2026; Accepted: 3 February 2026; Published: 9 February 2026

MONITORING OF ANTIBIOTIC RESIDUES IN BROILER CHICKEN LIVERS IN TABANAN REGENCY, BALI

Pemantauan Residu Antibiotik pada Hati Ayam Broiler di Kabupaten Tabanan, Bali

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How to cite: Arinata IKT, Tenaya IWM, Agustina KK, Besung INK, Sudira IW, Suardana IBK. 2026. Monitoring of antibiotic residues in broiler chicken livers in Tabanan regency, Bali. *Bul. Vet. Udayana* 18(1): 244-254. DOI: https://doi.org/10.24843/bulvet.2026.v18.i01.p24

Abstract

Antibiotic residues in animal products can pose health risks, including antimicrobial resistance, allergies, and toxicity. The use of antibiotics in inappropriate doses, either excessive or insufficient, can cause antibiotic residues in animal products, especially in the liver, milk, and meat. Broiler chicken livers have a higher risk of residue accumulation than other organs due to their role in drug metabolism. This study aims to determine the presence or absence of antibiotic residues in broiler chicken livers in relation to the level of knowledge and attitudes of farmers regarding the use of antibiotics administered during the broiler chicken rearing process in Tabanan Regency, Bali. This study is observational with a purposive sampling method that requires 20 broiler chicken liver samples, and data on farmers' knowledge and attitudes regarding antibiotic use is obtained directly through interviews from broiler chicken farms. Antibiotic residues in broiler chicken liver samples will be tested using a screening test based on the Kirby-Bauer method at the Denpasar Veterinary Public Health Laboratory. The research data was tabulated and analyzed descriptively, both qualitatively and quantitatively. The test results showed that all broiler chicken liver samples were negative for antibiotic

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residues. These results were supported by the farmers' high level of knowledge regarding the proper use of antibiotics, compliance with withdrawal periods, and the application of doses that were generally in accordance with recommendations. Although all farmers still reported using antibiotics during rearing, the pattern of administration was monitored and found to be controlled and not excessive. It is hoped that these good attitudes and practices can be maintained in order to ensure the safety of poultry-derived food.

Keywords: Antibiotics, attitude, chicken, knowledge, liver, residue

Abstrak

Residu antibiotik dalam produk hewani dapat menimbulkan risiko kesehatan, termasuk resistensi antimikroba, alergi, dan toksisitas. Penggunaan antibiotik dengan dosis yang tidak tepat, kelebihan atau kekurangan dosis dapat menyebabkan residu antibiotik dalam produk hewani terutama hati, susu dan daging. Hati ayam broiler memiliki risiko akumulasi residu lebih tinggi dari organ lainnya, karena perannya dalam metabolisme obat. Penelitian ini bertujuan untuk mengetahui ada atau tidaknya residu antibiotik pada hati ayam broiler yang dikaitkan dengan tingkat pengetahuan dan sikap peternak terkait penggunaan antibiotik yang diberikan saat proses pemeliharaan ayam broiler di Kabupaten Tabanan, Bali. Penelitian ini adalah observasional dengan metode purposive sampling yang memerlukan 20 sampel hati ayam broiler, serta data pengetahuan dan sikap peternak terkait penggunaan antibiotik diperoleh langsung dengan wawancara dari peternakan ayam broiler tersebut. Residu antibiotik pada sampel hati ayam broiler akan diuji dengan uji tapis (*screening test*) berdasarkan metode *Kirby-Bauer* di Laboratorium Kesehatan Masyarakat Veteriner Balai Besar Veteriner Denpasar. Data hasil penelitian ditabulasi dan dianalisis secara deskriptif kualitatif maupun kuantitatif. Hasil pengujian menunjukkan seluruh sampel hati ayam broiler negatif terhadap residu antibiotik, hasil ini didukung oleh tingginya pengetahuan peternak mengenai penggunaan antibiotik yang tepat, ketaatan terhadap masa henti obat, serta penerapan dosis yang umumnya sesuai dengan anjuran. Walaupun seluruh peternak masih melaporkan penggunaan antibiotik selama pemeliharaan, pola pemberiannya terpantau terkontrol dan tidak berlebihan. Sikap dan praktik yang baik ini diharapkan dapat terus dipertahankan guna menjaga keamanan pangan asal unggas.

Kata kunci: Antibiotik, ayam, hati, pengetahuan, residu, sikap.

INTRODUCTION

The demand for animal-based food in Indonesia continues to increase in line with population growth, improved nutritional knowledge, lifestyle changes, and improved community welfare. Data from the Central Statistics Agency shows that the consumption of meat, eggs, and milk is an important part of the Indonesian population's consumption patterns (Buletin Konsumsi Pangan, 2024). In line with this, national broiler chicken production has increased and is projected to reach 3.84 million tons in 2024, exceeding national consumption needs (Kementerian Pertanian, 2024). To support productivity, broiler chicken farms still widely use antibiotics for therapy, prophylaxis, and feed additives (Purnawarman & Efendi, 2020).

Tabanan Regency is one of the main centers of broiler chicken farming in Bali Province, with the highest production contribution compared to other regencies/cities. The population and production of broiler chickens in this region show high and sustainable figures (Yuliari *et al*., 2021; Zulfa *et al*., 2015). The high intensity of production has the potential to increase the use of antibiotics, which, if not in accordance with the rules, can cause residues in animal-derived food products. Uncontrolled use of antibiotics can cause residues in animal products and have negative impacts on human health, such as allergic reactions, intestinal microflora disorders,

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and antimicrobial resistance (Loh *et al*., 2018; Tumanduk *et al*., 2023; Widhi & Saputra, 2021). Several studies have reported antibiotic residues in broiler chicken products that exceed the maximum residue limit (MRL), particularly in the tetracycline and macrolide groups (Aniza *et al*., 2019; Marlina *et al*., 2015).

Broiler chicken liver has a high tendency to store antibiotic residues because it functions as the main organ for drug metabolism and detoxification (Etikaningrum & Iwantoro, 2017; Siswanto & Sulabda, 2018). Various studies in Indonesia show a high prevalence of antibiotic residues in broiler chicken livers (Marlina *et al*., 2015; Widiastuti *et al*., 2004). Given that chicken liver is widely consumed by the public, including vulnerable groups, regular testing for antibiotic residues is essential. Based on this background, this study aims to identify the presence of antibiotic residues in broiler chicken livers in Tabanan Regency.

RESEARCH METHODS

Research Object

The research object used was broiler chicken farmers spread across Tabanan Regency in Bali Province. A total of 20 broiler farms in Tabanan Regency were visited to collect data using guided interview techniques with questionnaires. Samples were taken from each farm, consisting of one 100-gram liver sample, which was wrapped, labeled, and stored in a cool box with ice packs until it was examined in the laboratory.

Research Design

The methods used in this study included field observation, sampling, and laboratory testing of samples. This study used an observational design. Samples were taken using a cross-sectional method accompanied by survey data from respondents through guided interviews using questionnaires. The broiler chicken farm sample population was determined using purposive sampling, which is a sampling method carried out deliberately based on certain considerations in accordance with the research objectives. The selected broiler chicken liver samples came from several broiler chicken farms in Tabanan Regency that met the inclusion criteria.

According to Guest *et al*., (2006), in studies with a relatively homogeneous population, 12 respondents are often sufficient to achieve data saturation. This is reinforced by the opinions of Marshall, (1996) and Creswell, (2013), who state that in qualitative or exploratory research, a sample size of 15 to 30 participants is generally sufficient, depending on the complexity of the issue and the purpose of the analysis. Based on these considerations, the sample size used in this study was 20 broiler chicken livers, which were selected purposively and considered representative of the general conditions in the Tabanan Regency, Bali. Data on the maintenance process, antibiotic use, and farmers' understanding were collected using interview techniques and direct assessment at the location.

Meanwhile, the livestock product, namely broiler chicken liver, was taken directly from the farms that had been interviewed. The liver samples were then labeled, wrapped in sample plastic, and stored in a cool box containing blocks of ice. Next, the samples were taken to the testing laboratory for examination of antibiotic residue content. Based on ISO 17604 regarding the transportation temperature and storage time of meat samples taken from carcasses, the storage temperature is 2±2 ºC and testing is carried out within a maximum of 24 hours. To determine the presence of antibiotics or residues in broiler chicken meat products, all samples obtained were examined using the BioAssay method based on the Kirby-Bauer method. The work was carried out at the Veterinary Public Health Laboratory of the Disease Investigation Center (BBVet).

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Criteria

The inclusion criteria in this study included broiler farms that were in production with chickens ready for harvest and broiler farms that were managed for commercial purposes. The exclusion criteria included broiler farms that did not give permission for interviews and broiler farms that were not in production or had chickens that were not yet ready for harvest.

Research Procedures

The antibiotic residue screening test is a test to identify antibiotic residues in samples. This test is carried out by inhibiting the growth of microorganisms in agar media by the antibiotic residues present in the sample. The test results can be seen from the formation of an inhibition zone around the disc. This test must be carried out aseptically in a sterile room.

Antibiotic Residue Examination

Residue testing is carried out using a screening test method that refers to Indonesian National Standard (SNI) 7424:2008. Antibiotic residue detection is performed using the Bioassay Screening Test method on Muller Hinton Agar (MHA) media that has been inoculated with test bacteria. The test bacteria used are Bacillus cereus ATCC 11778 for tetracycline antibiotics, Geobacillus stearothermophylus ATCC 7953 for penicillin antibiotics, Kocuria rhizophila ATCC 9341 for macrolide antibiotic residues, and Bacillus spszizenii ATCC 6633 for aminoglycoside antibiotics.

A total of 1 mL of test bacterial culture was mixed with 100 mL of liquefied media. For the Bacillus stearothermophilus ATCC 7953 media, 2.5% of a 2% dextrose solution was added. Next, 8 mL of media containing the test bacteria was pipetted and placed in each Petri dish according to the type of antibiotic group and triplicate replicates (three Petri dishes) were performed. Take 75 µl of the homogenized sample solution and drop it onto a disc of paper, then place it in the medium. Drop the reference standard solution as a positive control and the buffer solution as a negative control. Place each Petri dish on a flat surface in a room at room temperature for one hour.

The dishes were then incubated for 16-18 hours, for macrolides and aminoglycosides at a temperature of 36 °C ± 1 °C, for tetracyclines at a temperature of 30 °C ± 1 °C, and for penicillins at a temperature of 55 °C ± 1 °C. The test result was considered positive if an inhibition zone formed from the edge of the paper disc on which the sample was dropped, and negative if no inhibition zone formed.

Data Analysis

The research data were tabulated and analyzed descriptively, both qualitatively and quantitatively. The percentage of antibiotic use and the presence or absence of residues were analyzed descriptively and quantitatively in numerical form, while the level of knowledge and attitudes of broiler farmers regarding antibiotic use were analyzed descriptively and qualitatively by presenting the data in narrative form.

RESULTS AND DISCUSSION

Results

Antibiotic residue testing of all liver samples yielded negative results. A total of 20 liver samples obtained from broiler chicken farms in each subdistrict in Tabanan Regency, Bali, showed no antibiotic residues. The survey results showed that all respondents (100%) used antibiotics in their chicken farms. Various types of antibiotics were used, with Amoxicillin and Ciprofloxacin being the most commonly used at 14.7% each. The use of Norfloxacin and

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Tylosin was found to be 11.8%, while Erythromycin, Doxycycline, Enrofloxacin, and Oxytetracycline were used at 8.8% each. The antibiotics Tiamulin and Sulfadimidine were used at a lower percentage, namely 5.9%.

No antibiotics were found to be used to stimulate growth in healthy chickens. However, all respondents (100%) administered antibiotics as a preventive measure before symptoms appeared. Antibiotics were mostly administered when the chickens were 1 day old (95.2%) with the longest administration period being 3 days (75%). Most respondents (75%) consulted a veterinarian before administering antibiotics, while the rest did not (25%). The choice of antibiotic type was mostly determined by partner companies (87%), while veterinary advice was only a reference for 13% of respondents. The determination of antibiotic dosage was mostly based on the antibiotic packaging information (45%), while 17.5% was determined by veterinarians. Antibiotics were mostly obtained from partner companies (62.5%), followed by veterinarians (25%) and stores (12.5%).

In practice, antibiotics were mostly administered independently by farmers (85%), while only 15% of respondents called a veterinarian. The most common method of administering antibiotics is through drinking water (86.9%), while injections are administered to a small proportion of respondents (13.1%). When treatment does not show results, 90% of respondents switch to a different type of antibiotic. Farmers' level of knowledge about antibiotic use in general is relatively high. All respondents are aware that inappropriate antibiotic dosing can cause residues and resistance. Most respondents (95%) also knew about the withdrawal time before chickens are marketed. All respondents (100%) were aware of the dangers of food products containing antibiotic residues, while 85% of respondents knew about the importance of veterinary supervision. A total of 95% of respondents knew that antibiotics are only effective against bacterial infections and are not effective against viral infections. However, 25% of respondents were still unaware that antibiotic use can disrupt the balance of gastrointestinal microflora, and another 25% were unaware of government regulations governing the use of antibiotics in poultry farming.

Discussion

The broiler chicken farms surveyed in this study were dominated by small-scale businesses with between 5,001 and 50,000 broiler chickens, while the rest were micro-scale businesses with less than 5,000 chickens. These farms were spread across various subdistricts in Tabanan Regency, Bali, and no farms were found that fell into the medium-scale category (50,001–1,000,000). The characteristics of this business scale became an important context in the discussion of husbandry practices and drug use at the farmer level. Under these conditions, it was found that none of the samples showed antibiotic residues. The selection of the liver as the target organ in the antibiotic residue test in this study was based on its role as the main metabolic organ responsible for the biotransformation and detoxification of drugs. The journey of antibiotics in the animal's body after absorption, most antibiotics will pass through the liver via the portal vein system before being distributed to other tissues, so that initial exposure and the highest accumulation of residues generally occur in this organ (Beyene, 2015). The liver also serves as the most sensitive indicator for detecting the presence of residues, as antibiotic concentrations in this organ tend to be higher than in muscle or fat tissues (Donato *et al*., 2024). In addition, international institutions such as Codex Alimentarius and the European Food Safety Authority (EFSA) recommend the liver and kidneys as standard target tissues in the monitoring of veterinary drug residues, given their important physiological role in metabolism and their relevance to human food safety (EFSA, 2017). The use of liver as a test sample in this study was considered the most representative for evaluating the potential for antibiotic residues in broiler chicken products.

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A total of 20 broiler chicken liver samples tested using the Bioassay (Kirby–Bauer) method showed negative results for the presence of antibiotic residues. This result was confirmed by the absence of a clear zone around the disc paper on the agar medium. This is in line with the findings Yani *et al*., (2022) which state that antibiotic residues can be detected if there is an inhibition of microorganism growth, indicated by the formation of a clear zone around the disc, so that the absence of this zone indicates the absence of antibiotic residues in the sample. Balouiri *et al*., (2016) reported that cases of antibiotic residues in Indonesia during the 2011–2016 period showed a downward trend. Several studies even found no antibiotic residues in poultry products, although there were still findings of tetracycline residues at relatively high levels, namely 4.1% and 4.17%. These findings are in line with a study in East Selemadeg, Tabanan, Bali in 2022, which also reported no antibiotic residues from the tetracycline, aminoglycoside, macrolide, or penicillin groups in broiler chicken and duck meat samples (Permatasari *et al*., 2022). In addition, bioassay results on chicken meat from five broiler farms in Tabanan Regency also showed no tetracycline antibiotic residues. However, the study found weak and inconsistent antibiotic activity in feed and farm waste samples (Setiabudy *et al*., 2023).

Negative antibiotic residue test results in broiler chicken livers can be influenced by several important factors, one of which is the farmer's understanding of the appropriate use of antibiotics in accordance with the withdrawal time and the correct dosage. Withdrawal time is the interval between the last administration of the drug and when the livestock is declared safe for slaughter, which is when the drug level in the body has decreased to below the safe consumption limit (Beyene, 2015). In practice, antibiotics are generally administered to chickens at Day Old Chick (DOC), which is well before harvest time. Thus, the drug has sufficient time to degrade and be eliminated from the body before the chickens are slaughtered, leaving no residue in organs such as the liver. This is in line with the findings (Roth *et al*., 2019) which state that antibiotics are administered at the beginning of broiler chicken rearing because the chickens' immune systems are not yet fully developed, making them susceptible to infection by environmental pathogens such as Escherichia coli, Clostridium spp. and Salmonella spp., which remain at risk of entering through equipment, labor, feed, drinking water, or biological vectors even when biosecurity and closed housing systems are strictly implemented. Antibiotics are used as a preventive measure to suppress the bacterial population in the early phase before the immune response from vaccination is fully formed.

Similar results were also reported by Aarestrup (2015), who stated that the administration of antibiotics from the early stages of rearing is a common practice in many developing countries because it is considered to reduce mortality and improve production performance. This is in line with the 2017 Indonesian Ministry of Agriculture Survey report, which shows that 81.4% of poultry farmers use antibiotics for disease prevention, 30.2% for treatment, and 0.3% still use them for growth promotion purposes (EFSA, 2017). Suandy *et al*., (2024) also confirm that high broiler mortality in the first week of rearing contributes to increased antibiotic use during this period to reduce mortality rates. The high level of antibiotic use is also influenced by the ease of access to these drugs, while consultation services or recommendations from veterinarians are still difficult to obtain (Coyne *et al*., 2020). Another contributing factor is the broiler chicken harvesting pattern, which is generally carried out at around 30 days of age. The long interval between antibiotic administration and slaughter allows the drugs to break down optimally and not accumulate in the tissue (Yani *et al*., 2022). Several studies also report that antibiotics for disease prevention are usually administered at 2–4 days or 8–10 days of age, further increasing the likelihood of antibiotic degradation before the chickens reach slaughter age (Bimantara *et al*., 2021). Physiologically, the liver plays a major role in the biotransformation of antibiotics into compounds that are more easily excreted. This process

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involves two stages, namely phase I metabolism by enzymes such as cytochrome P450, followed by phase II in the form of conjugation reactions that increase the water solubility of these compounds (Iacopetta *et al*., 2023). The resulting metabolites generally have lower biological activity than the parent drug, such as in the macrolide group, which produces metabolites with much lower antibacterial activity (Wang *et al*., 2024). Because these metabolites are inactive and more easily eliminated through urine or bile, antibiotic residues become difficult to detect. In addition, the bioassay method used in this study has lower sensitivity than chemical analysis techniques such as HPLC or LC-MS/MS. Bioassay methods are qualitative and can only detect residues above a certain sensitivity range (1.25 µg/kg for beta-lactams, 30 µg/kg for tetracyclines, and 100 µg/kg for aminoglycosides/macrolides), so residues below these levels are often undetectable. In contrast, quantitative techniques such as HPLC-DAD can measure antibiotic residues in the range of 5.37–55.4 µg/kg LOD (limit of detection) and 17.9–185 µg/kg LOQ (limit of quantification) depending on the type of antibiotic analyzed, and LC-MS/MS can achieve even more sensitive LOD values for various classes of antibiotic residues. If the residue concentration is below the detection limit, screening tests may yield negative results even though there are still very small traces of residue (Park *et al*., 2025). In addition to biological and technical aspects, the level of knowledge of farmers is also a crucial factor that supports the absence of antibiotic residues in broiler chicken livers. The interview results show that all farmers (100%) understand what antibiotics are and their function in poultry farming, and know that the use of antibiotics in inappropriate doses can cause residues and antimicrobial resistance. As many as 95% of farmers also knew the withdrawal time rules before chickens were marketed, 100% knew that food products containing antibiotic residues were harmful to health, and 95% understood that antibiotics were only effective for bacterial infections, not viruses. This is in line with the findings Efendi *et al*., (2022) which show that 90% of small-scale broiler farmers in Bogor are aware of the antimicrobial withdrawal period, confirming that knowledge of the withdrawal period is an important aspect of responsible antibiotic use practices to prevent residues in poultry products. Research in India reports that veterinarians consistently explain the importance of the withdrawal period to farmers every time they prescribe antibiotics, as part of efforts to ensure the proper use of antibiotics and prevent residues in livestock products (Sharma *et al*., 2024).

Additionally, 75% of farmers know that antibiotic use must be under the supervision of a veterinarian, although in practice only 15% actually call a veterinarian when using them. Understanding of the long-term effects of antibiotic use is also very good, with 100% of farmers stating that they are aware of this. This high level of knowledge indicates that farmers tend to be more cautious in their use of antibiotics, thereby minimizing the risk of residues in chicken tissue. These findings are in line with a study published in Acta Veterinaria Indonesiana, which reported that farmers with a good understanding of the function, dosage, and withdrawal period of antibiotics tend to apply more rational and recommended drug use practices (Purnawarman & Efendi, 2020).

CONCLUSIONS AND SUGGESTIONS

Conclusion

Based on the results of antibiotic residue testing using the Kirby–Bauer bioassay screening method, all broiler chicken liver samples from Tabanan Regency, Bali, showed negative results, indicating that no antibiotic residues were detected in the samples tested. These findings suggest that if antibiotic residues are still present, they are in such low quantities that they cannot be detected by the testing method used. Thus, the broiler chicken livers tested can be declared safe for consumption based on the testing method used in this study. The level of knowledge and attitude of broiler chicken farmers in Tabanan Regency, Bali, regarding the use

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of antibiotics is fairly good. Most farmers understand the rules regarding the withdrawal period before chickens are marketed and are aware of the importance of using antibiotics under the supervision of a veterinarian. This understanding and positive attitude play a role in reducing the risk of antibiotic residues in poultry products, even though the use of antibiotics in the early stages of rearing is still relatively high as a disease prevention measure.

Suggestion

Broiler chicken farmers in Tabanan Regency are advised to continue to use antibiotics wisely by paying attention to the dosage and withdrawal period as recommended by veterinarians. Additionally, ongoing guidance and education on rational antibiotic use are needed to ensure consistent implementation at the farmer level. Regular monitoring of antibiotic residues using more sensitive testing methods is also recommended to strengthen poultry product safety oversight and support efforts to prevent antimicrobial resistance.

ACKNOWLEDGEMENTS

The author would like to thank all those who have helped in writing this literature review article so that it could be completed successfully.

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Table

Table 1. Results of Antibiotic Residue Testing in Broiler Chicken Livers in Tabanan Regency, Bali.

No.	District	Number of Samples	Results
1.	Marga	2	Negative
2.	Pupuan	3	Negative
3.	Kediri	1	Negative
4.	Tabanan	2	Negative
5.	Baturiti	2	Negative
6.	Kerambitan	2	Negative
7.	Penebel	3	Negative
8.	Salamadeg	1	Negative
9.	Salamadeg Timur	2	Negative
10.	Salamadeg Barat	2	Negative

Terakreditasi Nasional Sinta 4, berdasarkan Keputusan Direktur Jenderal Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021